



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K	A2	(11) International Publication Number: WO 00/03677 (43) International Publication Date: 27 January 2000 (27.01.00)						
<p>(21) International Application Number: PCT/IL99/00387</p> <p>(22) International Filing Date: 14 July 1999 (14.07.99)</p> <p>(30) Priority Data: 125336 14 July 1998 (14.07.98) IL</p> <p>(71) Applicants (for all designated States except US): YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM [IL/IL]; Jabotinsky Street 46, 92182 Jerusalem (IL). HADASIT MEDICAL RESEARCH SERVICES & DEVELOPMENT LIMITED [IL/IL]; P.O. Box 12000, Kiryat Hadassah, Jerusalem 91120 (IL).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): GOLOMB, Gershon [IL/IL]; Tirosch Street 20, 90435 Efrat (IL). DANEBERG, Haim [IL/IL]; 47 Dulcin Street, Jerusalem 96407 (IL).</p> <p>(74) Agent: REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel Aviv (IL).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published Without international search report and to be republished upon receipt of that report.</p>						
<p>(54) Title: TREATMENT OF RESTENOSIS</p> <div data-bbox="332 1150 1356 1480"> <table border="1"> <caption>NEointima/Media Ratio Data</caption> <thead> <tr> <th>Group</th> <th>NEointima/Media Ratio (approx.)</th> </tr> </thead> <tbody> <tr> <td>CLODRONATE LIPOSOMES</td> <td>0.3</td> </tr> <tr> <td>CONTROL LIPOSOMES</td> <td>1.5</td> </tr> </tbody> </table> </div> <p>(57) Abstract</p> <p>Bisphosphonate (BP), pyrophosphate (PP) a complex of BP or PP, a polymer of BP or PP or a pharmaceutically acceptable salt or ester thereof, are used for the prevention or treatment of vascular restenosis.</p>			Group	NEointima/Media Ratio (approx.)	CLODRONATE LIPOSOMES	0.3	CONTROL LIPOSOMES	1.5
Group	NEointima/Media Ratio (approx.)							
CLODRONATE LIPOSOMES	0.3							
CONTROL LIPOSOMES	1.5							

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

TREATMENT OF RESTENOSIS

FIELD OF THE INVENTION

The present invention is concerned with compositions capable of preventing, inhibiting or reducing restenosis (sometimes referred to in the art as "accelerated arteriosclerosis" and "post-angioplasty narrowing").

5

PRIOR ART

The following references are considered to be pertinent for the purpose of understanding the background of the present invention.

- 10 1. Waller, B.F., Orr, C.M, VanTassel J., *et al. Clin-Cardiol.* **20**(2):153-60, (1997).
2. Anderson, W.D, King, S. Br., *Curr-Opin-Cardiol.*, **11**(6):583-90, (1996).
- 15 3. Moorman, D.L., Kruyer, W.B., Jackson, W.G., *Aviat-Space- Environ-Med.* **67**(10):990-6, (1996).
4. Laurent S, Vanhoutte P, Caverio I, *et al., Fundam. Clin. Pharmacol.*, **10**(3):243-57, (1996).
- 20 5. Walsh, K., Perlman, *Semin-Interv-Cardiol.*, **1**(3):173-9, (1996).
6. Schwartz, R.S., *Semin-Interv-Cardiol.*, **2**(2):83-8, (1997).
- 25 7. Allaire, E, Clowes, A.W. *Ann. Thorac. Surg.*, **63**:582-591, (1997).
8. Hamon. M., Bauters, C., McFadden, E.P, *et al., Eur. Heart J.*, **16**:33s-48s, (1995).

9. Gottsauner-Wolf, M., Moliterno, D.J., Lincoff, A.M., Topol, E.J., *Clin. Cardiol.*, **19**:347-356, (1996).
10. Herrman, J.P.R., Hermans, W.R.M., Vos, J., Serruys, P.W., *Drugs*, **46**:18-52, (1993).
11. Leclerc, G., Voisine, P., Bouchard, M., Fleser, A., Martel, R. *Elsevier Science*, 722-724, (1995).
12. Topol, E., *The NY Academy of Sciences*, 225-277, (1997).
13. Fleisch, H., in: Bisphosphonates in bone disease. Parthenon Publishing Group Inc., pp. 184-186 (1997).
14. Mönkkönen, J., Taskinen, M., Auriola, S., Urtti, J. *Drug Target*, **2**:299-308, (1994).
15. Kramsche, D.M., and Chan, C.T., *Circ. Res.*, **42**:562-572, (1978).
16. Braunwald, E. *Heart Disease* in: A textbook of cardiovascular medicine; 5th Ed., W.B. Saunders Company: Philadelphia, (1997).
17. Gennaro Alfonso, R. Remington, in: The Science and Practice of Pharmacy, Mack Publishing, Easton PA, 19th ed., (1995).
18. Mönkkönen, J., and Heath, T.D., *Calcif. Tissue Int.*, **53**:139-145, (1993).
19. M. Donbrow in: Microencapsulation and Nanoparticles in Medicine and Pharmacy, CRC Press, Boca Raton, FL, p. 347.

The above references will be acknowledged in the text below by indicating their number (as shown in brackets) from the above list.

BACKGROUND OF THE INVENTION

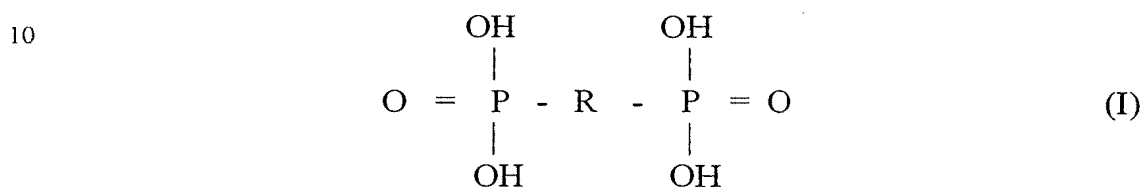
Over the past decade, mechanical means of achieving revascularization of obstructive atherosclerotic vessels have been greatly improved. Percutaneous transluminal coronary angioplasty (PTCA) procedures include but are not limited to, balloon dilatation, excisional atherectomy, endoluminal stenting, rotablation

and laser ablation. However, revascularization induces thrombosis, and neointimal hyperplasia, which in turn cause restenosis in a substantial proportion of coronary arteries after successful balloon angioplasty and in aortacoronary saphenous vein bypass graft and other coronary grafts. Furthermore, intimal
5 hyperplasia causes restenosis in many superficial femoral angioplasties, carotid endarterectomies, and femoro-distal vein bypasses. The introduction of endovascular stents has contributed to reduce the incidence of restenosis, but this problem still remains significant⁽¹⁻⁹⁾. Despite extensive research on the incidence, timing, mechanisms and pharmacological interventions in humans and animal
10 models to date no therapy exists which consistently prevents coronary restenosis⁽¹⁰⁻¹²⁾. Compositions and methods for the reduction or prevention of restenosis are still today greatly desired.

Bisphosphonates (BP) (formerly called diphosphonates) are compounds characterized by two C-P bonds. If the two bonds are located on the same carbon
15 atom (P-C-P) they are termed geminal bisphosphonates. The BPs are analogs of the endogenous inorganic pyrophosphate which is involved in the regulation of bone formation and resorption. The term bisphosphonates is generally used for geminal and non-geminal bisphosphonates. The BPs and pyrophosphates may at times form together polymeric chains. BPs act on bone because of their affinity
20 for bone mineral and are potent inhibitors of bone resorption and ectopic calcification. BPs or pyrophosphate have been used clinically mainly as (a) antiosteolytic agents in patients with increased bone destruction, especially Paget's disease, tumor bone disease and osteoporosis; (b) skeletal markers for diagnostic purposes (linked to ^{99m}Tc); (c) Inhibitors of calcification in patients
25 with ectopic calcification and ossification, and (d) antitartar agents added to toothpaste⁽¹³⁾.

SUMMARY OF THE INVENTION

In accordance with the present invention, a BP or pyrophosphate (collectively herein: "*active ingredient*") is used for treatment or prevention of a vascular. The term biphosphonate (BP) as used herein denotes both geminal and
 5 non-geminal biophosphonates. The term "*active ingredient*" encompasses in its scope also polymeric chains of the BPs or pyrophosphate, particularly such chains consisting of up to 40 BP monomers. Preferred active ingredients are compounds of the following formula (I)



15

wherein R represents O or a CR₁R₂ group;

R₁ is H, OH or a halogen group; and

R₂ is halogen; linear or branched C₁-C₁₀ alkyl or C₂-C₁₀ alkenyl optionally substituted by heteroaryl or heterocyclil C₁-C₁₀ alkylamino or C₃-C₈
 20 cycloalkylamino where the amino may be a primary, secondary or tertiary; -NHY where Y is hydrogen, C₃-C₈ cycloalkyl, aryl or heteroaryl; or R₂ is -SZ where Z is chlorosubstituted phenyl or pyridinyl;

The present invention thus provides the use of said active ingredient, a complex of said active ingredient or a pharmaceutically acceptable salt or ester
 25 thereof, for the preparation of a medicament for the prevention or treatment of vascular restenosis.

The present invention also provides a method of treatment of restenosis, comprising administering to an individual in need an effective amount of said active ingredient, a complex thereof or a pharmaceutically acceptable salt or ester
 30 thereof.

The present invention still further provides a pharmaceutical composition for the prevention or treatment of restenosis, comprising, as an active ingredient, an effective amount of said active ingredient, a pharmaceutically acceptable free acid, a complex or a salt thereof, optionally together with a pharmaceutically acceptable carrier or diluent. A particularly preferred carrier is a liposome preparation.

The term "*effective amount*" means to denote an amount of the active ingredient which is effective in achieving the desired therapeutic result, namely prevention or reduction of vascular restenosis. The effective amount may depend on a number of factors including: weight and gender of the treated individual; the type of medical procedure, e.g. whether the vascular restenosis to be inhibited is following balloon angioplasty, balloon angioplasty followed by deployment of a stent, etc.; the mode of administration of the active ingredient (namely whether it is administered systemically or directly to the site); the type of carrier being used (e.g. whether it is a carrier that rapidly releases the active ingredient or a carrier that releases it over a period of time); the therapeutic regime (e.g. whether the active ingredient is administered once daily, several times a day or once every few days); clinical factors influencing the rate of development of restenosis such as diabetes, smoking, hypercholesterolemia, renal diseases, etc.; anatomical factors such as whether there is severe preangioplasty stenosis, total occlusion, left anterior descending coronary artery location, saphenous vein graft lesion, long lesions, multivessel or multilesion PTCA; on the dosage form of the composition; etc. Moreover, procedural variables may also have bearing on the dosage such as greater residual stenosis following PTCA, severe dissection, intimal tear, appropriate size of balloon, and the presence of thrombus. The artisan, by routine type experimentation should have no substantial difficulties in determining the effective amount in each case.

The invention is applicable for the prevention, reduction or treatment of vascular restenosis and mainly, but not limited to, coronary restenosis after angioplasty. Vascular restenosis results from various angioplasty procedures

including balloon angioplasty, intravascular stent implantation or other methods of percutaneous angioplasty (including angioplasty of coronary arteries, carotid arteries, and other vessels amenable for angioplasty) as well as for restenosis resulting from vascular graft stenosis (e.g. following by-pass surgery)⁽¹⁶⁾. In addition, the invention is also applicable for use for prevention, reduction or treatment of vascular restenosis in peripheral arteries and veins.

One exemplary application of the invention is to prevent and treat in-stent restenosis. It is a widely acceptable medical procedure to deploy a stent within a blood vessel within the framework an angioplastic procedure, to support the walls of the blood vessel. However, very often restenosis occurs notwithstanding the presence of the stent within the blood vessel. In accordance with the invention the above noted active ingredient may be administered, either systemically or directly to the site, in order to prevent or inhibit such restenosis. Potentially said active ingredient may be formulated in a manner allowing its incorporation onto the stent which will in fact yield administration of said active ingredient directly at the site. The active ingredient may be formulated in that manner, for example, by including it within a coating of the stent. Examples of coating are polymer coatings, e.g., made of polyurethane, or a gel.

The active ingredient used in accordance with the invention may be formulated into pharmaceutical compositions by any of the conventional techniques known in the art (see for example, Alfonso, *et al.*, 1995⁽¹⁷⁾). The compositions may be prepared in various forms such as capsules, tablets, aerosols, solutions, suspensions, as a coating of a medical device such as a stent (see above), etc. In addition, the pharmaceutical compositions of the invention may be formulated in a form suitable for topical administration with a carrier or in a delivery form permitting penetration of the active ingredient through the skin into the body in order to act systemically. The preferred administration form in each case will depend on the desired delivery mode, which is usually that which is the most physiologically compatible in accordance with the patient's condition, other

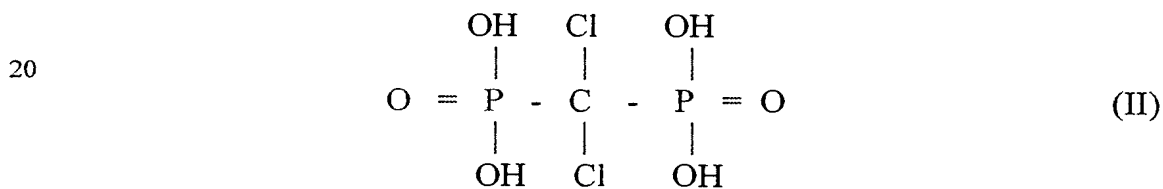
therapeutic treatments which the patient receives, etc. In accordance with a preferred embodiment of the invention, said active ingredient is formulated into a particulate form. This may be achieved by encapsulating or impregnating the active ingredient into particles, e.g. polymeric particles, lipid vesicles, or liposomes.

5 Furthermore, such particles may be particles of polymerized active ingredient (see below). Particularly preferred are liposomal preparations of said active ingredient. The liposomes may be prepared by any of the methods known in the art (regarding liposome preparation methods see Mönkkönen *et al.*, 1994⁽¹⁴⁾ and Mönkkönen *et al.*,⁽¹⁸⁾). The liposomes may be positively charged, neutral or negatively charged

10 (negatively charged liposomes being currently preferred), may be single or multilamellar. At times, compositions comprising a combination of free active ingredient (i.e. non-encapsulated) and said active ingredient encapsulated within liposomes may also be used.

By a preferred embodiment of the invention, the active ingredient is BP

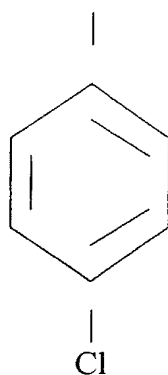
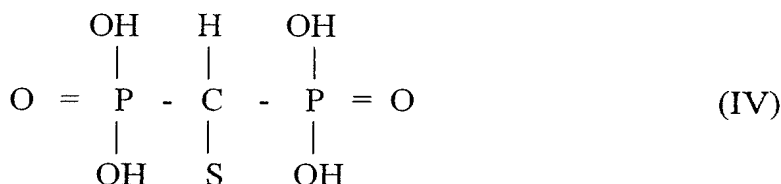
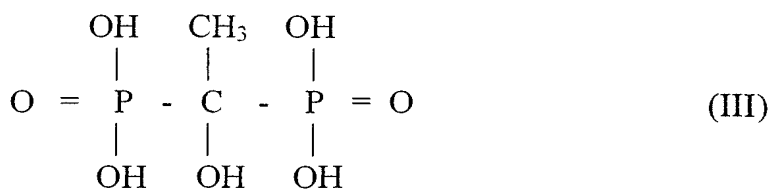
15 preferably selected from the group of such compounds which are capable of undergoing intracellular metabolism. One preferred active ingredient for this group is the compound clodronate⁽¹³⁾ having the following formula (II):



25 Clodronate, was previously described for use in the treatment of hypercalcemia resulting from malignancy in the treatment of tumor associated osteolysis⁽¹³⁾, and as an inhibitor of macrophages^(14,18).

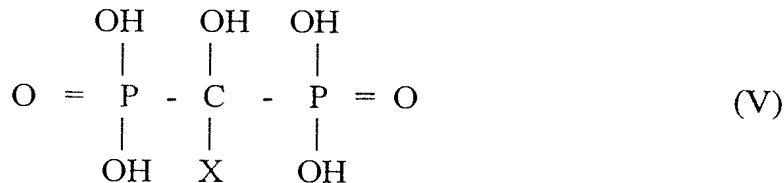
Other preferred active ingredients of this group are Etidronate and Tiludronate having the following formulae (III) and (IV) respectively:

30



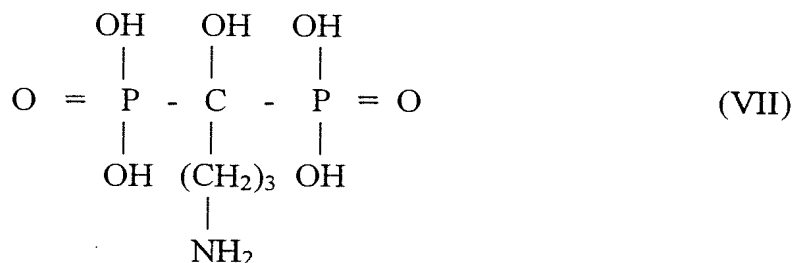
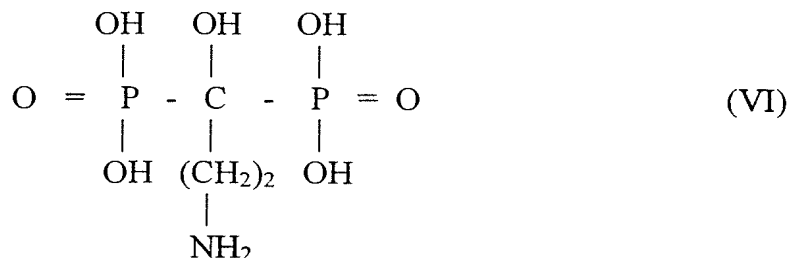
Additional BPs having activities similar to that of clodronate are also preferred in accordance with the invention. Such BPs may be selected on the basis of their ability to mimick biological activity of clodronate. This includes, for example: *in vitro* activity in inhibiting phagocytic activity of phagocytic cells, e.g. macrophages and fibroblasts; inhibition of secretion of IL-1 and/or IL-6 and/or TNF- α from macrophages; *in vivo* activity, e.g. the ability of the tested BP to prevent or reduce restenosis in an experimental animal model such as, for example, the rat or rabbit carotid catheter injury model described in Example 1 below, or porcine model of restenosis; etc.

Another preferred group of active ingredients in accordance with the invention are the amino-BPs and any other nitrogen-containing BPs having the following general formula (V)



wherein x represents C₁-C₁₀ alkylamino or C₃-C₈ cycloalkylamino, where the amino may be primary, secondary or tertiary; or x represents NHY where Y is hydrogen, C₃-C₈ cycloalkyl, aryl or heteroaryl

The BPs belonging to this group are believed not to be metabolized and have been shown at relatively low concentrations to induce secretion of the interleukin IL-1 and cause, at relatively high concentrations, apoptosis in macrophages⁽¹⁸⁾. Preferred BPs belonging to this group are for example, Pamidronate and Alendronate having the following formulae (VI) and (VII), respectively.

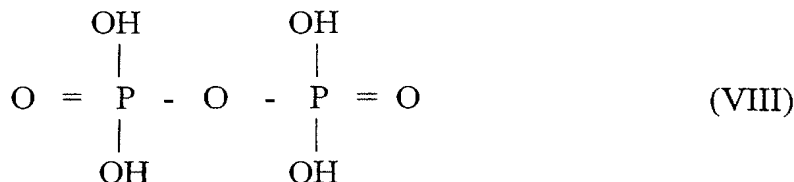


Although the geminal BPs are preferred BPs in accordance with the invention, non-geminal BPs, monophosphonates of BPs termed generally as

phosphonates may also be used as active ingredients in accordance with the invention.

Another preferred active ingredient is pyrophosphate having the formula (VIII):

5



10

Pyrophosphate is preferably formulated and administered in a liposome or a polymeric particle preparation.

The composition of the invention may comprise said active ingredient either
15 in their free acid form, complexed with metal cations such as calcium, magnesium or organic bases or may be in the form of salts or esters or they may be polymerized to yield polymers of up to 40 monomers. The salts may be sodium, potassium, ammonium or calcium salts or salts formed with any other suitable basic cation (e.g. organic amino compounds). The salts or polymers may be in a micronized
20 particulate form having a diameter within the range of about 0.01-10 μm , preferably within a range of about 0.1-5 μm . The active ingredients in the compositions of the invention being either in their free acid form or salt form may be with or without water of crystallization (hydrous and anhydrous).

By a preferred embodiment, the active ingredient is encapsulated within a
25 liposome prepared by any of the methods known in the art. Suitable liposomes in accordance with the invention are preferably non toxic liposomes such as, for example, those prepared from phosphatidyl-choline phosphatidylglycerol, and cholesterol, e.g. as described below. In many cases, use of the active ingredient encapsulated in a liposome results in enhanced uptake of the active ingredient by
30 cells via endocytosis^(14,18) (such uptake may play a role in the therapeutic effect). The diameter of liposomes used in the examples below was between 0.15 to

300 nm. However, this is non-limiting, but merely an example, and liposomes of other size ranges may also be used.

In addition, the active ingredient may be encapsulated or embedded in inert polymeric particles such as, for example, any of the microcapsules, nanocapsules, nanoparticles, nanospheres, microspheres, microparticles, etc. known in the art⁽¹⁹⁾. The release of the active ingredient from such particles may be a controlled release which results, in some cases, in prolonged and enhanced effect and uptake of said active ingredient.

The pharmaceutical carrier or diluent used in the composition of the invention may be any one of the conventional solid or liquid carriers known in the art. A solid carrier for example may be lactose, sucrose, talc, gelatin, agar, etc. Where the carrier is liquid carrier it may be for example peanut oil, phospholipids, water etc. Where the solid carrier is used for oral administration, the composition may be in the form of a tablet, in the form of a hard capsule (e.g. a gelatin capsule), in the form of powder or in pellet form. Where a liquid carrier is used, the preparation may be in the form of a syrup, emulsion, liposomes, soft gelatin capsule or sterile injectable liquid such as a liposome preparation, an aqueous or non-aqueous liquid suspension or a solution⁽¹⁷⁾.

The composition of the invention used for injection may be selected from emulsions, solutions, suspensions, colloidal solutions containing suitable additives, etc.

The compositions of the invention may be administered by any route which effectively transports the active compound to the appropriate or desirable site of action. By a preferred embodiment of the invention the modes of administration are intravenous (i.v.) and intra-arterial (i.a.) (particularly suitable for on-line administration). Other modes of administration include intramuscular (i.m.) or subcutaneous (s.c.). Such administration may be bolus injections or infusions. The compositions may also be administered locally to the diseased site of the artery, for example, by means of a suitable oozing/sweating balloon known in the art. Another

mode of administration may be by perivascular delivery, coating of the delivery system on a balloon or a stent or by any of the other methods of cardiovascular drug delivery systems known in the art. Combinations of any of the above routes of administration may also be used in accordance with the invention.

5 The dosage of the active ingredient to be used also depends on the specific activity of the used active ingredient, on the mode of administration (e.g. systemic administration or local delivery), the form of the active ingredient (e.g. whether it is in the form of a drug, in a form of a polymer, whether it is encapsulated in a particle such as a liposome, etc.), and other factors as known *per se*.

10 In accordance with a preferred embodiment of the invention, treatment of an individual with the active ingredient may be for the purpose of preventing restenosis before its occurrence. For prevention, the active ingredient may be administered to the individual before angioplasty procedure, during the procedure or after the procedure as well as combination of before, during and after procedural
15 administration.

 In accordance with another embodiment of the invention, the active ingredient is administered to an individual suffering from restenosis for the purpose of reducing or treating restenosis. In such a case, the active ingredient may also be administered to the individual at different periods of time after restenosis is
20 discovered, either alone or in combination with other kinds of treatments.

 In addition, the active ingredient may be administered before any other conditions which may yield accelerated arteriosclerosis, as well as acutely after the process has begun to inhibit further development of the condition.

25 **BRIEF DESCRIPTION OF THE DRAWING**

Figs. 1-3 are bar graphs of results demonstrating the effect of Clodronate encapsulated in liposomes on the reduction of restenosis in an experimental rat carotid catheter injury model as compared to the effect of control liposomes which did not contain clodronate on the same rats. In these figures:

carotid catheter injury model as compared to the effect of control liposomes which did not contain clodronate on the same rats. In these figures:

Fig. 1 shows the extent of mean luminal intimal formation and mean neointima to media ratio in rats treated with Clodronate containing liposomes as compared to rats treated with control liposomes;

Fig. 2 shows the % stenosis in rats treated with Clodronate containing liposomes as compared to the % stenosis in rats treated with control liposomes; and

Fig. 3 shows the extent of medial area as an indirect index of smooth muscle cell viability and determined as the difference between the total arterial area and the original lumen area in rats treated with Clodronate containing liposomes as compared to rats treated with control liposomes only.

EXAMPLES

The invention will now be demonstrated by way of non limiting example with reference to the accompanying drawing:

Example 1

Materials and Methods

Liposomes of clodronate

Stock solutions of clodronate were prepared by dissolving the drug in deionized water at a concentration of 0.11 M, pH = 7.

Liposome preparation

38.9 mg of distearoylphosphatidylglycerol (DSPG), 118.5 mg of distearoyl- phosphatidylcholine (DSPC) and 38.7 mg of cholesterol were accurately weighed and dissolved in 20 ml of chloroform: methanol (9:1) in a round bottom vial. The vial was gently warmed. The solvent was then evaporated in rotavapor. 20 mls of hydrated diisopropylether were then added and the vial was put into a water bath until the contents were dissolved. 8 mls of the

clodronate solution prepared as described above, were then added, and the solution was sonicated at 55°C for a period of 45 mins. The organic phase was then evaporated in rotavapor (55°C, 100 rpm). Similarly, other drug-containing liposomes can be prepared.

5

Purification of prepared liposomes

A Sephadex gel was prepared by dissolving 2.6 gr of Sephadex G-50 in 40 mls of water and stabilizing over night. The column was rinsed with 100 mls of buffer (50 mM Mes + 50 mM HEPES + 75 mM NaCl, pH 7.2). The liposomes
10 were applied to the column and the column was rinsed with the buffer. The liposome is seen as a band which can be followed in the column by its color. About 20 drops were collected from the column into each tube.

Animals

15 Animals were obtained and housed in the animal facilities of the Faculty of Medicine, The Hebrew University of Jerusalem conforming to the standards for care and use of laboratory animals of the Hebrew University of Jerusalem. Male rats of Sabra strain weighing 350-420g were used. The animals were fed standard laboratory chow and tap water ad libitum. All *in vivo* experiments were
20 made under general anaesthetesia achieved with 80 mg/kg ketamine and 5mg/kg xylazine administered i.p.

Rat carotid catheter injury model

The distal left common and external carotid arteries were exposed through
25 a midline incision in the neck. The left common carotid artery was denuded of endothelium by the intraluminal passage of a 2F balloon catheter introduced through the external carotid artery. The catheter was passed three times with the balloon distended sufficiently with saline to generate a slight resistance. The

catheter was then removed and the external carotid artery was ligated, and the wound was closed with surgical staples.

Seven rats served as the control group, and 6 rats as the treated group (randomly chosen). Liposomal clodronate was injected I.V. to the "treated group" one day prior to the arterial injury (6 mg of clodronate per rat) and repeated on day 6. In the control group similar injections were administered but with "empty" liposomes (no clodronate).

All animals were sacrificed 14 days after injury by an overdose of pentobarbital. Arteries were perfusion-fixed with 150ml of 4% formaldehyde solution pH 7.4 at 100 mm Hg. The right atrium was dissected and an 18G catheter connected to the perfusion system was inserted in the left ventricle. The arterial segments were dissected, cut, gently separated from the polymer, and postfixed for at least 48hrs. in the same fixative solution. The arterial segments were embedded in paraffin and cut at 8-10 sites 600 μ m apart, and sections of 6 μ m were mounted and stained with Verhoeff's elastin stain for histologic examination.

Morphometric analysis

The slides were examined microscopically by an investigator blinded to the type of the experimental group. Six to 8 sections in each slide were evaluated by computerized morphometric analysis and the averaged section data were further used as a representative of a whole slide for comparisons between groups. The residual lumen, the area bounded by the internal elastic lamina (original lumen), and the area circumscribed by the external elastic lamina ("total arterial area") were measured directly. The degree of neointimal thickening was expressed as the ratio between the area of the neointima and the original lumen (% stenosis), and as the ratio between the neointimal area to the area of the media (N/M). The medial area, an indirect index of SMC viability, was determined as the difference between the total arterial area and the original lumen area.

Results

The surgical procedure and treatment did not cause mortality or apparent morbidity of the animals.

As seen in Fig. 1 the extent of mean neointimal formation and mean
5 neointima to media ratio (N/M) following treatment with
clodronate-encapsulated in liposomes was significantly reduced. The N/M ratio
in clodronate treated rats was 0.28 ± 0.23 as compared to 1.42 ± 0.26 in the
control group (mean \pm SD, $p < 0.01$). Similarly as seen in Fig. 2, significant
inhibition of % stenosis was achieved in the treated group: 9.8 ± 7.76 vs. $41.53 \pm$
10 7.9 , treated and control groups, respectively (mean \pm SD, $p < 0.01$). There were
no apparent systemic side effects nor any effects on somatic growth as seen in
Fig. 3.

Conclusion: Treatment of rats with clodronate-containing liposomes significantly
reduces restenosis seen as neointimal formation following balloon-injury of the
15 carotid artery.

Example 2

Materials and Methods

The antirestenotic effects of liposomal clodronate injections were studied in
20 the balloon-injured rat and atherosclerotic rabbit carotid arterial models. The rats
were treated by clodronate-containing liposomes, empty liposomes (control), and
clodronate in solution (additional control). The dose of clodronate injected was 1.5
and 15 mg/kg administered one day before procedure (-1) and/or on day 6 (+6) post
injury. The rabbits (following 30 days of atherosclerotic diet) were treated one day
25 prior to balloon angioplasty by liposomal clodronate (10 mg/kg). The lumen,
neointimal, medial and vessel areas and volumes were measured in the treated and
control animal groups by digital planimetry of histological sections, at 14 and 30
days post injury in the rat and rabbit models, respectively.

Results

The results are shown in the following Table 1.

Treatment type	Animal (No.)	Dosing days	N/M	% stenosis
Empty liposomes	Rat (20)	-1, +6	1.62 ± 0.1	44.5 ± 2.0
Clodronate	Rat (10)	-1, +6	1.2 ± 0.2	40.2 ± 7.2
Liposomal clodronate 15 mg/kg	Rat (10)	-1	0.45 ± 0.1	15.4 ± 3.4
Liposomal clodronate 15 mg/kg	Rat (10)	-1, +6	0.35 ± 0.07	12.0 ± 2.3
Empty liposomes	Rabbit (10)	-1	1.94 ± 0.3	86.6 ± 4.0
Liposomal clodronate 10 mg/kg	Rabbit (10)	-1	1.11 ± 0.2	71.5 ± 2.1
Silica particles 1000 mg/kg	Rat (10)	-1	0.8 ± 0.1	24.2 ± 3.8

As can be seen no significant differences were found between treatments with empty liposomes, and free clodronate in solution (see table), exhibiting marked neointimal formation. The extent of mean neointimal formation, mean neointima to media ratio (N/M), and % stenosis following treatment with clodronate-laden liposomes was significantly reduced. The medial area was not affected by the various treatments indicating no deleterious effects on quiescent cells. Moreover, there were neither apparent systemic side effects nor any effects on bone and somatic growth. Significantly more potent treatments, with no significant difference between them, were the 1 x 15 mg/kg (-1) and 2 x 15 mg/kg (-1, and +6) injections. Similar findings of no adverse effects were observed in the rabbits' study. Liposomal clodronate was significantly effective in reducing neointimal formation and % stenosis.

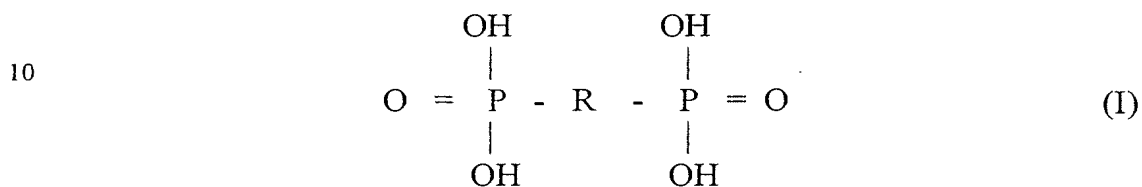
Injection of silica particles also reduces intimal formation (Table 1). This effect could be attributed to the known inhibiting effect of silica on macrophages.

The results show that treatment by clodronate-containing liposomes significantly reduces neointimal formation following balloon-injury in both rat and rabbit models. There were neither apparent systemic and local side effects nor any

effects on somatic growth. It should be noted that although BP are known as affecting bone, no effects on the bone nor on calcium and phosphorus levels in bone and blood were observed, following treatment with liposomal preparation of clodronate.

CLAIMS:

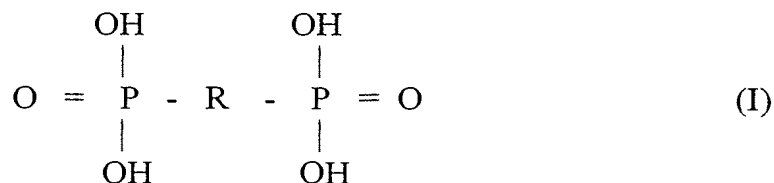
1. Use of an active ingredient being a bisphosphonate (BP), or pyrophosphate (PP) a complex of BP or PP, a polymer of BP or PP or a pharmaceutically acceptable salt or ester thereof, for the preparation of a pharmaceutical composition
5 for the prevention or treatment of vascular restenosis.
2. Use according to Claim 1, wherein said active ingredient has the general formula (I):



- 15 wherein R represents O or a CR₁R₂ group;
R₁ is H, OH or halogen group; and
R₂ is halogen; linear or branched C₁-C₁₀ alkyl or C₂-C₁₀ alkenyl optionally substituted by heteroaryl or heterocyclil C₁-C₁₀ alkylamino or C₃-C₈ cycloalkylamino where the amino may be a primary, secondary or tertiary; -NHY
20 where Y is hydrogen, C₃-C₈ cycloalkyl, aryl or heteroaryl; or R₂ is -SZ where Z is chlorosubstituted phenyl or pyridinyl;
or a complex of said BP or a pharmaceutically acceptable salt or ester thereof, for the preparation of a pharmaceutical composition for the prevention or treatment of vascular restenosis.
- 25 3. Use according to Claim 2, wherein said active ingredient is selected from the group consisting of clodronate, etidronate, tiludronate, pamidronate, alendronate, pyrophosphate or pharmaceutically acceptable salts or esters thereof.
4. Use according to any one of Claims 1 to 3, wherein said restenosis is arterial restenosis.
- 30 5. Use according to any one of Claims 1-4, wherein said use comprises incorporating said active ingredient into or encapsulated within particles.

6. Use according to Claim 5, comprising encapsulating said active ingredient with liposomes.
7. Use according to any one of Claims 1-5, wherein said use comprises embedding said active ingredient into polymeric particles.
- 5 8. Use according to any one of Claims 1-7, comprising preparing a composition for systemic administration.
9. Use according to any one of Claims 1-4, comprising preparing a composition for incorporation into a stent.
10. A method of treatment of restenosis, comprising administering to an individual in need an effective amount of an active ingredient selected from the group consisting of bisphosphonate (BP), pyrophosphate (PP), a complex of BP or PP, a polymer of BP or PP or a pharmaceutically acceptable salt or ester thereof.
- 10 11. A method according to Claim 10, comprising administering to the individual an active ingredient, having a compound having the following formula (I):

15



20

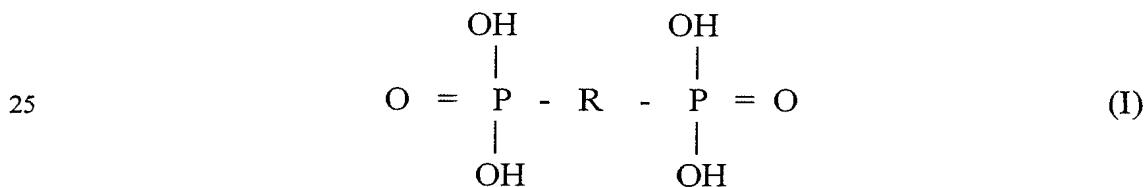
wherein R represents O or a CR₁R₂ group;

R₁ is H, OH or halogen group; and

wherein R₁ is H, OH or halogen group; and

- 25 R₂ is halogen; linear or branched C₁-C₁₀ alkyl or C₂-C₁₀ alkenyl optionally substituted by heteroaryl or heterocyclil C₁-C₁₀ alkylamino or C₃-C₈ cycloalkylamino where the amino may be a primary, secondary or tertiary; -NHY where Y is hydrogen, C₃-C₈ cycloalkyl, aryl or heteroaryl; or R₂ is -SZ where Z is chlorosubstituted phenyl or pyridinyl;
- 30 or a complex, salt, ester or polymer thereof.

12. A method according to Claim 11, wherein said active ingredient is clodronate, etidronate, tiludronate, pamidronate, alendronate, pyrophosphate or pharmaceutically acceptable salts or esters thereof.
13. A method according to any one of Claims 10-12, wherein said restenosis is coronary restenosis.
14. A method according to any one of Claims 10-12, comprising administering to the individual particles formed of or having incorporated therein said active ingredient.
15. A method according to Claim 14, comprising administering liposomes comprising said active ingredient.
16. A method according to any one of Claims 10 to 15, wherein said active ingredient is administered systemically.
17. A method according to any one of Claims 10 to 16, wherein said active ingredient is administered locally to a needing site.
18. A pharmaceutical composition comprising as an active ingredient, a bisphosphonate (BP), a pyrophosphate (PP), a complex of BP or PP, a polymer of BP or PP or a pharmaceutically acceptable salt or ester thereof together with a pharmaceutically acceptable carrier or diluent for the prevention or treatment of vascular restenosis.
19. A pharmaceutical composition according to Claim 16, wherein said active ingredient has the general formula (I):



wherein R represents O or a CR₁R₂ group;

R₁ is H, OH or halogen group; and

R₂ is halogen; linear or branched C₁-C₁₀ alkyl or C₂-C₁₀ alkenyl optionally substituted by heteroaryl or heterocyclil C₁-C₁₀ alkylamino or C₃-C₈ cycloalkylamino where the amino may be a primary, secondary or tertiary; -NHY where Y is hydrogen, C₃-C₈ cycloalkyl, aryl or heteroaryl; or R₂ is -SZ where Z is
5 chlorosubstituted phenyl or pyridinyl.

20. A composition according to Claim 19, wherein said active ingredient is clodronate, etidronate, tiludronate, pamidronate, alendronate, pyrophosphate or pharmaceutically acceptable salts and esters of said compounds.

21. A pharmaceutical composition according to any one of Claims 18-20,
10 wherein said restenosis is coronary restenosis.

22. A pharmaceutical composition according to any one of Claims 19-21, wherein said composition comprises particles made of or having incorporated therein said active ingredient.

23. A pharmaceutical composition according to Claim 22, wherein said particles
15 are liposome particles encapsulating such active ingredient.

24. A pharmaceutical composition according to Claim 22, wherein said active ingredient is embedded in a polymeric particle.

25. A pharmaceutical composition according to any one of Claims 18 to 24, wherein said active ingredient is a micronized particulate form.

1/1

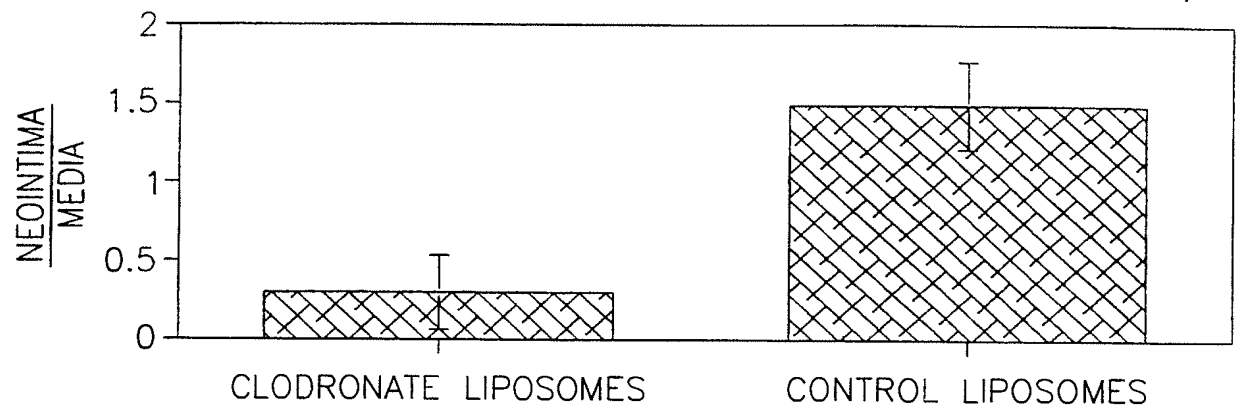


FIG.1A

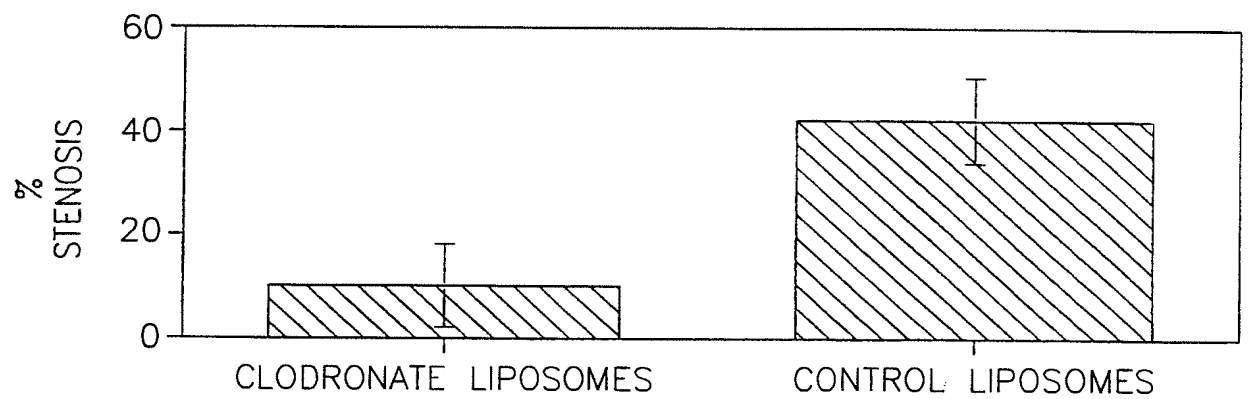


FIG.2

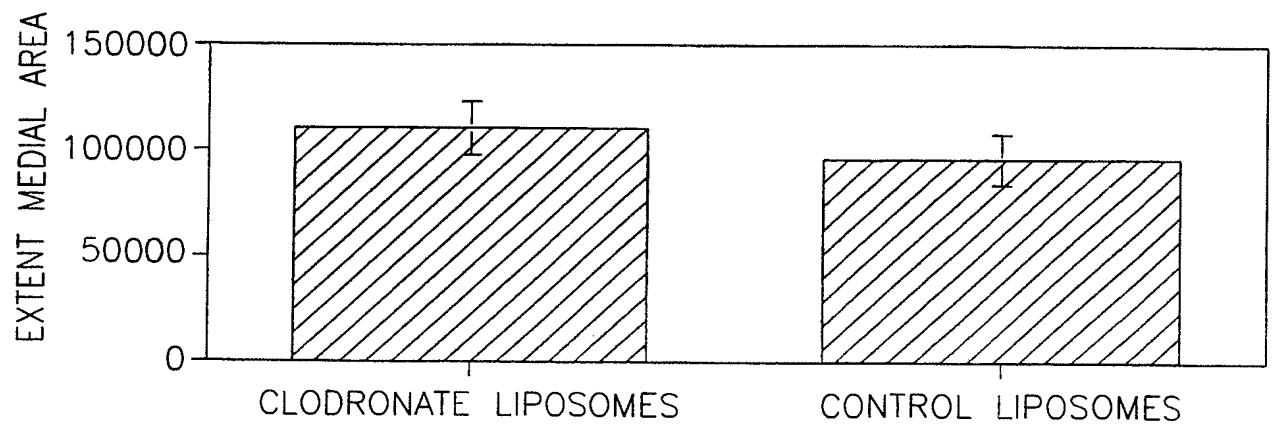


FIG.3



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 31/66	A3	(11) International Publication Number: WO 00/03677 (43) International Publication Date: 27 January 2000 (27.01.00)						
<p>(21) International Application Number: PCT/IL99/00387</p> <p>(22) International Filing Date: 14 July 1999 (14.07.99)</p> <p>(30) Priority Data: 125336 14 July 1998 (14.07.98) IL</p> <p>(71) Applicants (for all designated States except US): YISSUM RESEARCH DEVELOPMENT COMPANY OF THE HEBREW UNIVERSITY OF JERUSALEM [IL/IL]; Jabotinsky Street 46, 92182 Jerusalem (IL). HADASIT MEDICAL RESEARCH SERVICES & DEVELOPMENT LIMITED [IL/IL]; P.O. Box 12000, Kiryat Hadassah, Jerusalem 91120 (IL).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): GOLOMB, Gershon [IL/IL]; Tirosh Street 20, 90435 Efrat (IL). DANEBERG, Haim [IL/IL]; 47 Dulcin Street, Jerusalem 96407 (IL).</p> <p>(74) Agent: REINHOLD COHN AND PARTNERS; P.O. Box 4060, 61040 Tel Aviv (IL).</p>		<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report.</p> <p>(88) Date of publication of the international search report: 4 May 2000 (04.05.00)</p>						
<p>(54) Title: USE OF BISPHOSPHONATES IN THE TREATMENT OF VASCULAR RESTENOSIS</p>								
<table border="1"> <caption>Bar Chart Data</caption> <thead> <tr> <th>Group</th> <th>Neointima / Media Ratio (approx.)</th> </tr> </thead> <tbody> <tr> <td>CLODRONATE LIPOSOMES</td> <td>0.3</td> </tr> <tr> <td>CONTROL LIPOSOMES</td> <td>1.5</td> </tr> </tbody> </table>			Group	Neointima / Media Ratio (approx.)	CLODRONATE LIPOSOMES	0.3	CONTROL LIPOSOMES	1.5
Group	Neointima / Media Ratio (approx.)							
CLODRONATE LIPOSOMES	0.3							
CONTROL LIPOSOMES	1.5							
<p>(57) Abstract</p> <p>Bisphosphonate (BP), pyrophosphate (PP) a complex of BP or PP, a polymer of BP or PP or a pharmaceutically acceptable salt or ester thereof, are used for the prevention or treatment of vascular restenosis.</p>								

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Larvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

INTERNATIONAL SEARCH REPORT

Int. l. Application No

PCT/IL 99/00387

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/66

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 99 38998 A (MERCK AND CO. INC.) 5 August 1999 (1999-08-05) page 2, line 16 - line 25 page 12, line 30 -page 16, line 24; claims 8,11,12 ---	1-4, 10-13, 18-21
P,X	WO 98 31359 A (MERCK AND CO. INC.) 23 July 1998 (1998-07-23) page 3, line 18 - line 23 page 26, line 7 -page 27, line 6 page 29, line 13 - line 31 ---	1-8, 10-24
X	WO 97 43437 A (THE UNIVERSITY OF SHEFFIELD) 20 November 1997 (1997-11-20) page 20, line 5-15 page 27, line 10 -page 29, line 5 --- -/--	1-5,8, 10-22,25

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

8 February 2000

Date of mailing of the international search report

29/02/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Mair, J

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 99/00387

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO 97 33552 A (WALLACE TECHNOLOGIES INC.) 18 September 1997 (1997-09-18)</p> <p>page 3, line 21 - line 25 page 6, line 4 - line 16 page 9, line 11 -page 10, line 13</p> <p>---</p>	1-5, 8-14, 16-22
X	<p>DAOUD, A.S. ET AL: "The effect of ethane-1-hydroxy-1,1-diphosphonate (EHDP) on necrosis of atherosclerotic lesions" ATHEROSCLEROSIS, vol. 67, no. 1, 1987, pages 41-48, XP000874378 the whole document</p> <p>---</p>	1-4,8, 10-12, 16,18-20
X	<p>WAGNER, W.D. ET AL: "Contrasting effects of ethane-1-hydroxy-1,1-diphosphonate (EHDP) on the regression of two types of dietary-induced atherosclerosis" ATHEROSCLEROSIS, vol. 27, no. 4, 1977, pages 419-436, XP000874381 the whole document</p> <p>---</p>	1-4,8, 10-12, 16,18-20
X	<p>WEBB, C.L. ET AL: "Inhibition of bioprosthetic heart valve calcification with aminodiphosphonate covalently bound to residual aldehyde groups" THE ANNALS OF THORACIC SURGERY, vol. 46, no. 3, 1988, pages 309-316, XP000874363 the whole document</p> <p>---</p>	1-3,7, 10-14, 17-22,24
X	<p>BELLAH, R.D. ET AL: "Idiopathic arterial calcification of infancy: Prenatal and postnatal effects of therapy in an infant" THE JOURNAL OF PEDIATRICS, vol. 121, no. 6, 1992, pages 930-933, XP000874377 the whole document especially page 933, lefthand column, line 46</p> <p>---</p>	1,10,18
X	<p>SHIOI, A. ET AL: "Beta-Glycerophosphate accelerates calcification in cultured bovine vascular smooth muscle cells" ARTERIOSCLEROSIS, THROMBOSIS AND VASCULAR BIOLOGY, vol. 15, no. 11, 1995, pages 2003-2009, XP000874393 abstract page 2003, left-hand column, line 10-13 page 2007; figure 8</p> <p>---</p> <p>-/--</p>	1-3, 10-12

INTERNATIONAL SEARCH REPORT

Int. l. Application No
PCT/IL 99/00387

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	PASPALIARIS, V. ET AL: "Clodronate inhibits contraction and prevents the action of L-type calcium channel antagonists in vascular smooth muscle" JOURNAL OF BONE AND MINERAL RESEARCH, vol. 6, no. 8, 1991, pages 835-841, XP000874364 the whole document ----	1-26
A	EP 0 339 237 A (SYMPHAR S.A.) 2 November 1989 (1989-11-02) the whole document ----	1-25
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US AN: 000076062622, XP002130040 abstract & KUNITOMO, M. ET AL: "Experimental induction of atherosclerosis in guinea-pigs fed a cholesterol vitamin D2 rich diet" FOLIA PHARMACOLOGICA JAPONICA, vol. 81, no. 4, 1983, pages 275-284, -----	1-25

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IL 99/00387

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim(s) 10-17
is(are) directed to a method of treatment of the human/animal
body, the search has been carried out and based on the alleged
effects of the compound/composition.
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such
an extent that no meaningful International Search can be carried out, specifically:
SEE FURTHER INFORMATION SHEET PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-2, 4-11, 13-19 and 21-25 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds mentioned in claims 3, 12 and 20 and the general idea underlying the application.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IL 99/00387

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9938998	A	05-08-1999	AU 2487899 A	16-08-1999
WO 9831359	A	23-07-1998	AU 6023198 A	07-08-1998
WO 9743437	A	20-11-1997	AU 2783397 A	05-12-1997
			EP 0952834 A	03-11-1999
WO 9733552	A	18-09-1997	AU 2580697 A	01-10-1997
			CA 2250295 A	18-09-1997
			CN 1217662 A	26-05-1999
			CZ 9802908 A	14-07-1999
			EP 0932399 A	04-08-1999
			NO 984210 A	11-11-1998
			PL 328807 A	15-02-1999
			US 5977163 A	02-11-1999
EP 339237	A	02-11-1989	CH 675422 A	28-09-1990
			AT 84040 T	15-01-1993
			AU 3224389 A	05-10-1989
			CA 1338495 A	30-07-1996
			CN 1036772 A, B	01-11-1989
			CN 1086820 A	18-05-1994
			DK 148189 A	17-10-1989
			ES 2011210 T	01-01-1994
			FI 891538 A, B,	01-10-1989
			GR 90300002 T	07-06-1991
			GR 3007442 T	30-07-1993
			HU 50482 A, B	28-02-1990
			IE 62692 B	22-02-1995
			IL 89649 A	31-07-1995
			JP 2022285 A	25-01-1990
			JP 8022865 B	06-03-1996
			KR 147830 B	17-08-1998
			NO 891317 A	02-10-1989
			NZ 228516 A	21-12-1990
			PH 27603 A	31-08-1993
			PT 90175 A, B	10-11-1989
			US 5043330 A	27-08-1991
			US 5128331 A	07-07-1992
			ZW 3789 A	23-08-1989